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<p>(54) Title: VIP₂ (VASOACTIVE INTESTINAL POLYPEPTIDE) RECEPTOR</p>		
<p>(57) Abstract</p> <p>The present invention provides a VIP₂ receptor gene (seq ID No.1), and polynucleotide probes specifically binding to this gene or to naturally occurring variants thereof, as well as polypeptide probes specifically binding to the receptor polypeptide or to naturally occurring variants thereof. Expression of the gene in a host is also useful for providing means for use in the evaluation of VIP₂ agonists and antagonists.</p>		

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0 VIP2 (VASOACTIVE INTESTINAL POLYPEPTIDE) RECEPTOR

Background of invention

Field of invention

This invention relates to the discovery of cDNA sequences encoding for a newly found member of the recently discovered
5 secretin family of G-protein linked neuropeptide-receptors, which is a receptor for vasoactive intestinal polypeptide (VIP), and the application of this discovery to inter alia
1) the development and use of nucleotide sequences derived from the receptor for the measurement (qualitative and
10 quantitative) and regulation of gene expression and 2) the development and use of VIP agonist and antagonist analogues for clinical and therapeutic use in the management of human
pituitary dysfunction, various cancers of the brain, pituitary, lung, adrenal, and reproductive system and VIP
15 related disorders of brain function, and other conditions involving VIP.

Description of the Prior art

Vasoactive intestinal polypeptide (VIP) has been identified and purified from small intestine on the basis of its potent
20 vasodilator activity[1, 2]. VIP has a number of actions in the periphery including vasodilatation, stimulation of electrolyte secretion and smooth muscle relaxation[3]. VIP is a member of a family of structurally related polypeptide hormones which include glucagon, glucagon-like peptide I
25 (GLP), peptide histidine isoleucine (PHI), secretin, pituitary adenylate cyclase activating polypeptide (PACAP) and growth hormone releasing hormone (GHRH). All of these peptides are thought to exert their actions through G-protein linked membrane receptors coupled to adenylate
30 cyclase. Recently, a receptor for VIP[4], has been cloned from rat lung[5] and from a human colon carcinoma cell line[4]. Receptors for glucagon[6-8], GLP[9], GHRH[10-12], secretin[13] and PACAP[14-16] have also been cloned.

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Together with receptors for calcitonin[17, 18] and parathyroid hormone (PTH)[19, 20] they form a protein family distinct from other G protein-linked receptors.

VIP has been shown to bind with high affinity to VIP
5 receptors in a variety of tissues. These binding sites are present in lung, liver and intestine, as well as several regions of the brain (e.g. cerebral cortex, hypothalamus and hippocampus[21, 22]). This receptor is probably identical to
10 a receptor known as the PACAP Type II receptor[23, 24] which recognises VIP, PACAP-27 and PACAP-38 with very similar affinities and may correspond to the previously cloned VIP receptor[4, 5] which has a similar tissue distribution[5]. This receptor is present in a variety of peripheral tissues including rat liver, rat lung, mouse splenocytes and human
15 small intestinal epithelium[23, 25-28]. There is some evidence for the existence of other types of VIP receptor in the brain and periphery[29]. Cross-linking studies have been used to identify VIP binding proteins in tissues with molecular weights ranging from 46,000 to 73,000 depending on
20 the tissue and species[30]. Studies with peptide analogs also suggest the existence of more than one pharmacologically distinct class of VIP receptor[31-35]. A class of VIP receptor, for which helodermin is the most potent ligand, has been identified in certain human cell
25 lines, including the lymphoblastic cell line SUP-T1[36, 37] the THP-1 monocyte/macrophage cell line[38] and NCI-H345 lung carcinoma cells[39]. Hill et al. [40] have distinguished two subtypes (or different functional states of a single subtype) of VIP receptor which differ in their
30 sensitivity to GTP analogs. In some brain regions, guanylyl-imidodiphosphate (GMPPNP) substantially inhibited VIP binding. In other regions, VIP binding was insensitive to GMPPNP. Both types of receptor are present in the mouse embryo[41], where they are differentially regulated by
35 treatment with a VIP antagonist.

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To date, however, there have been no reports of the sequencing of any gene encoding a second VIP receptor with a distinct and different molecular structure.

The initial step in VIP action centres on the binding of VIP
5 to a specific membrane bound receptor. Multiple steps are then involved in triggering the synthesis of intracellular cAMP (cyclic Adenosine Monophosphate) in tissues such as brain, pituitary, lung, pancreas, gastrointestinal tract, kidney, reproductive tract, blood vessels and various others
10 and in certain tumours. In the central nervous system, VIP may play a role in the pathogenesis of psychiatric, neurological and neuroendocrine disorders. VIP may also regulate cerebral energy metabolism[42] and neuronal survival[43]. VIP stimulates prolactin secretion from the
15 pituitary[44], catecholamine release from the adrenal medulla[45] and in the immune system it inhibits mitogen activated proliferation of T cells by inhibiting interleukin-2 production[46].

Knowledge of the structure and characteristics of VIP
20 receptors in the brain and pituitary and other tissues would also be helpful both in attempting to use VIP or its analogs in pharmacotherapy and in attempting to understand the possible roles of VIP in the body.

Thus there is a particular need for providing probes and
25 other assay materials and methods for use in the detection and/or identification, directly or indirectly, of abnormalities in VIP receptors, especially human VIP receptors and/or their expression, and/or the genes encoding them, as well as means for use in the evaluation of
30 potential VIP agonists or antagonists for use in the treatment of such abnormalities.

It is an object of the present invention to avoid or minimise one or more of the above problems or disadvantages.

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Summary of Invention

We have now succeeded in the cloning and expression of a specific, adenylate cyclase-linked VIP receptor from rat brain. We have named this receptor the VIP₂ receptor to distinguish it from the receptors cloned from rat by Ishihara et. al. [5] and from human by Sreedharan et. al. [4]. The latter two receptors probably represent species variants of one gene, which we will refer to as the VIP₁ receptor. The VIP₂ receptor was identified by PCR of rat pituitary cDNA using degenerate oligonucleotide primers corresponding to the third and seventh transmembrane domains of the secretin family of G-protein linked receptors. Full length cDNAs were isolated from an olfactory bulb cDNA library. The cDNA sequence for the novel VIP receptor is presented as the basis of this invention. The sequence is presented in interleaved format (see Fig. 1), and has been submitted to the EMBL/Gen Bank database under accession No. Z25885.

Thus we have shown for the first time that VIP receptor occurs naturally in at least two different forms with distinct tissue distributions and different molecular structures. This indicates the possibility of different physiological functions for the different receptors and the need for diagnostic means and methods for distinguishing these different forms, as well as the possibility of providing VIP₂ agonists and antagonists adapted for selective interaction with the newly discovered VIP₂ receptor, for example for the purposes of controlling blood pressure and/or renal function, as well as certain tumours. Given the co-existence of both VIP₁ and VIP₂, provision by means of the present invention of cloned VIP₂ receptor gene and systems for expression thereof, is particularly valuable in providing means for assaying VIP₂ - specific agonists and antagonists.

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In more detail, the present invention provides: a gene encoding VIP₂ receptor; and preferably a gene encoding VIP₂ receptor having substantially the amino acid SEQ ID No:1 disclosed herewith and includes genes encoding VIP receptor
5 which genes have substantial nucleotide sequence homology with the nucleotide sequence SEQ ID No:1 disclosed herein; and in particular the genes of the present invention in a form substantially free from other genes. It will be understood that the genes of the present invention may
10 include nucleic acid sequences (upstream and/or downstream of the receptor coding sequence) which are utilized in the expression of the gene such as promoter, operator, and terminator sequences as well as other sequences which do not inhibit its expression. Thus the expression "gene" includes
15 DNA (including cDNA) and/or RNA sequences as well as plasmid or viral "genes" containing the receptor gene and expression vectors for the gene.

With respect to the distribution of VIP₂ receptor in the body we have found that significant, and in some cases
20 particularly high, concentrations are to be found in inter alia: the supra-chiasmatic nucleus of the brain which is involved in the control of circadian (or diurnal) and other biorhythms including those involved with sleep and arousal, feeding, drinking, various endocrine functions, and
25 ovulation; the dorsal horn of the spinal cord and especially the substantia gelatinosa and Clark's column which are involved in the transmission and processing of pain; and tumours, especially gastro-intestinal and lung tumours.

In one aspect therefore the present invention provides new
30 methods and means based upon the newly discovered VIP₂ receptor nucleotide sequence for use in the clinical diagnosis and therapeutic management of those processes and abnormalities thereof that involve the action of VIP and the VIP₂ receptor, including but not exclusive of:

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Neurological and psychiatric disorders including schizophrenia, depression, Alzheimer's disease and Parkinson's disease; Brain tumours; Pituitary tumours and neuroendocrine disorders; Cardiovascular and inflammatory disorders; Disorders of fertility, control of fertility, testicular tumours; impotence directly related to failure to achieve erection; Disorders of the kidney; Phaeochromocytoma and other tumours of neuroendocrine origin; Lung tumours; Leukaemia, immune and inflammatory disorders; Gastro-intestinal Tumours; Pancreatic tumours, pancreatitis, cystic fibrosis; Diabetes; Disorders of foetal development; especially foetal brain development; and Degenerative disorders of the nervous system.

In addition the invention provides methods and means for use in the control and/or regulation of circadian (or diurnal) and other biorhythms including those involved with sleep and arousal, feeding, drinking, various endocrine functions, and ovulation; as well as of pain thereby providing valuable new alternative means for providing analgesia.

In the case of tumours there may be used anti-sense polynucleotides in order to restrict or inhibit the growth of tumours. Preferably there are used anti-sense polynucleotides capable of binding to part of the gene which includes the ATG (initiation) codon corresponding to the start of the VIP2 protein coding region.

In general the VIP2 receptor gene sequences are also useful for the design of oligonucleotide probes capable of specifically hybridising with the genes of the present invention, and for the synthesis of polypeptides which may be used in immunoassays. In addition parts of the cDNA sequence may be used to design and/or provide oligonucleotide probes for use in identifying human and other mammalian VIP receptor genes. Thus the present invention also specifically extends to a human gene DNA

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sequence which has been obtained directly or indirectly through recovery of a human gene by hybridisation thereof with a polynucleotide probe comprising a labelled DNA or RNA sequence capable of specifically hybridizing to a gene

5 encoding VIP2 receptor having the amino acid sequence of SEQ ID No. 1 disclosed herein. In general any part of the VIP2 receptor coding region of the gene may be used for this probe. Both oligonucleotide probes and the polypeptides may be useful for the diagnosis of VIP2 receptor abnormalities.

10 (It will be understood that references to oligonucleotide probes and the use thereof also include such probes as part of longer sequences). Polypeptides encoded within the cDNA sequences may also be used to raise antibodies against selected regions of normal or abnormal VIP2 receptor

15 polypeptide, which are particularly implicated in ligand binding i.e. the first, second, third and fourth extracellular domains of the VIP2 polypeptide (see fig 2); or in signal transduction i.e. the first, second, third and fourth intra-cellular domains, especially the main

20 cytoplasmic loop or third intracellular domain, most preferably in the region of the interface between the third intracellular domain and the sixth transmembrane region. e.g. one of the four extra-cellular domains identified hereinbelow, and for the purification of antibodies directed

25 against such regions. These antibodies may be useful in immunoassays for detecting normal or abnormal VIP₂ receptor in individuals.

Furthermore the invention provides screening means for use in the evaluation of new VIP₂ receptor agonists and

30 antagonists, comprising a cell transformed with a recombinant expression system comprising an open reading frame (ORF) of DNA derived from a VIP₂ receptor gene or VIP₂ receptor cDNA, said ORF being operably linked to a control sequence compatible with said cell, as well as such

35 expression systems per se.

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Thus the present invention further includes a method of producing receptor which method includes the step of expressing the genes of the present invention in a host, as well as VIP₂ receptor produced by such a method. Various
5 suitable hosts are known in the art though eukaryotic hosts are generally preferred, e.g. *Xenopus* oocytes and COS-7 cells. Prokaryotic hosts that may be used include *E. coli*. and *B. Subtilis*. Fungi e.g. yeast may also be used. For the purposes of developing VIP₂ agonist and antagonist
10 analogues, it is particularly preferred to use cloned cell lines expressing human VIP₂ receptor. Genes for human VIP₂ receptor can be isolated and sequenced by screening a human cDNA library preferably a human tumour (e.g. adrenal, pancreas, lung or brain) or normal adult or foetal human
15 brain, with the aid of oligonucleotide probes from the entire rat VIP₂ receptor sequence or parts thereof.

Thus the present invention also includes products and processes utilizing, directly or indirectly, human VIP₂ receptor sequences obtained in this way.
20 In particular the invention provides means for the evaluation of new VIP₂ receptor agonists and antagonists. These may employ expression systems of the invention directly or in some cases simply the membranes (with VIP₂ receptor) thereof. Conveniently the transfected or
25 transformed cells, or the membranes thereof, are exposed to a suitable ligand e.g. labelled VIP, and the potential agonist or antagonist and the effect of the latter on the binding of the ligand is monitored, e.g. by examining the variation of binding level with agonist/antagonist dosage.
30 Alternatively or additionally there may be monitored a natural or artificial intra-cellular messenger system such as cAMP production, inositol phosphate turnover, cell calcium concentration, or expression of an artificial gene containing an enzyme marker producing a colour or light
35 reaction etc.

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A suitable method of restriction enzyme analysis in this invention depends on Restriction Fragment Length Polymorphisms (RFLPs). A sample is taken from any suitable tissue such as blood. DNA is extracted from the cells in any
5 conventional way. It is then digested with an appropriate restriction enzyme e.g. one which cuts in CG-rich sequence. The fragments of different length are separated by gel electrophoresis in any conventional way. A restriction fragment pattern is generated. Probing of the fragments will
10 generally be necessary for clearer detection of the pattern and of the fragment(s) of interest, e.g. a fragment which extends from restriction sites "n" to "n + 2" (where "n" denotes any arbitrary number), seemingly not being restricted at the normal site "n + 1" lying between "n" and
15 "n + 2" due to an abnormality at the "normal" restriction site. Alternatively, a polymorphism might generate restriction enzyme sites and thereby given rise to a plurality of shorter fragments where the normal DNA provides longer ones. Whether it is appropriate to probe for long or
20 short fragments will therefore depend on the circumstances of the polymorphism. In some instances, the probe will extend outside the region designated.

Although the RFLP method is one method of assay, it cannot be ruled out that direct hybridisation of probes to the
25 genomic region will be of interest. Thus suitable biopsy or other samples can be subjected to cloning techniques, to isolate a library of genomic DNA, or PCR of genomic DNA or cDNA. Clones containing the gene can be amplified by Polymerase Chain Reaction (PCR) and probes complementary to
30 the said region used directly on PCR products, which need not be first restricted by enzymes.

It will be appreciated, therefore, that the cDNA of the invention also has uses in assays which are not of the RFLP type. Accordingly, the polynucleotides per se are part of
35 this invention, as 'intermediates' suitable (when labelled)

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for use as probes. Both double-stranded and single-stranded polynucleotides are included as well as sense and anti-sense forms. Suitable polynucleotide probes may be oligonucleotides of from 10 to 50, preferably from 16 to 30 nucleotides in length. Shorter probes are unlikely to be sufficiently specific for the sequence of interest. Longer polynucleotide probes of from 100 to 500 nucleotides or more may also be used with longer ones (up to 2000 or more nucleotides) being useful e.g. for chromosomal in-situ hybridisation as further described hereinbelow. Preferably the probes relate to parts of the polynucleotide sequence corresponding to one or more domains, or portions thereof, of the VIP2 polypeptide, which are particularly implicated in ligand binding i.e. the first, second, third and fourth extracellular domains of the VIP2 polypeptide (see Figs. 1 and 2); or in signal transduction i.e. the first, second, third and fourth intra-cellular domains, especially the main cytoplasmic loop or third intra-cellular domain, most preferably in the region of the interface between the third intracellular domain and the sixth transmembrane region. The probe will usually be of DNA or RNA and labelled in any suitable manner e.g. by labelling with an enzyme, radioisotope, fluorescent, luminescent, or chemiluminescent labels or biotinylation.

25 The fragments are probed under any appropriate conventional hybridisation conditions, the fragments being conveniently first transferred to a filter. The complexes thus formed are detected by autoradiography or other detection means appropriate to the particular kind of label used.

30 Abnormalities in the polynucleotide sequence of restriction fragments of the genomic DNA or cDNA coding for VIP₂ receptor which are as small as single-point mutations can also be detected by means of Temperature Gradient Gel Electrophoresis in which a temperature gradient is superimposed, parallel to or transversely of, the electrical

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field in gel electrophoresis. The method is based on the fact that the temperature of denaturation of double stranded (ds) DNA is altered by changes in polynucleotide sequence. Furthermore, partial denaturation of a DNA duplex causes a change in electrophoretic mobility. Further details of this technique are described in the literature by Reisner et al, 1989 (56) and, Birmse et al, 1990 (57).

We have now further found that the chromosomal location for the VIP2 receptor gene is at 7q36.3 and thus the present invention now also provides further diagnostic means for use in the detection of conditions associated with VIP₂ abnormalities, which comprises one or more of hybridisation of a polynucleotide probe of the invention with the 7q36.3 chromosome region; and examination of the 7q36.3 chromosome region for gross abnormalities. Major defects in the 7q36.3 chromosomal region have been found to be associated with the birth defect holoprosencephaly, especially the type 3 form, and thus the present invention also provides a method of diagnosis for holoprosencephaly comprising the steps of collecting a foetal sample and examining the chromosomal content thereof by one or more of the techniques described hereinbefore so as to detect the presence of defects or irregularities in the 7q36.3 chromosomal region.

The VIP₂ receptor cDNA cloned and sequenced is shown in Fig. 1A. Fig. 2 illustrates schematically the predicted transmembrane, intracellular and extracellular domains of the receptor molecule. The transmembrane domains consist of seven stretches of hydrophobic in nature amino acids which span the membrane. The extracellular domain consists of four hydrophilic in nature amino acid stretches which exist exterior to the cell membrane. This region is believed to be important for the recognition of specific ligands. The intracellular domain consists of four hydrophilic stretches of amino acids which are thought to be involved in signal transduction.

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It will be appreciated that abnormality in human VIP₂ receptor and/or its expression, may be "assayed" in a number of ways. Thus the DNA encoding the VIP₂ receptor may itself be assayed for the presence or absence of abnormalities or
5 the VIP₂ receptor polypeptide may be assayed for such purposes, where this is actually expressed.

The former case generally involves the use of labelled polynucleotide probes to hybridise with DNA within the region coding for VIP₂ receptor polypeptide for the purposes
10 of indicating the presence or absence of particular polynucleotide sequences. In the latter case antibody probes are used to form antigen-antibody complexes with regions of the expressed polypeptide for the purposes of indicating the presence or absence of particular polypeptide sequences.
15 It will be understood that once more or less common or typical abnormalities have been specifically identified e.g. by initially probing with 'normal' polynucleotide and then sequencing, polynucleotide probes can be synthesized or otherwise produced with sequences corresponding to or
20 complementary to the "abnormal" sequences, to allow screening of tissue samples for specific VIP₂ receptor gene abnormalities. In the case of gene abnormalities such as the complete or substantial absence of the VIP₂ receptor gene, then this may be detected by failure to yield any
25 binding with suitable probes or absence of any product from PCR amplification using polynucleotide probes complementary to substantially spaced apart portions of the gene.

In the case of assays of the polypeptide itself, suitable stretches of amino acids based on the cDNA sequence
30 information provided by the present information, may be synthesised on a peptide synthesiser. These peptides would generally have a length of from 10 to 50, preferably 15 to 30, amino acids but could be even shorter or longer. Alternatively the complete VIP₂ receptor polypeptide or
35 fragments thereof may be expressed in a suitable eukaryotic

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or prokaryotic host such as E. Coli using an appropriate vector. Polyclonal antibodies to these peptides may be produced by conventional approaches such as the immunisation of host animals (rabbit, goat etc.) with said peptides, 5 optionally conjugated to a protein carrier such as thyroglobulin, and recovery of the desired antibody material therefrom. Monoclonal antibodies could also be raised using conventional monoclonal antibody-production procedures.

It will also be understood that "assay" may be either 10 qualitative or quantitative (e.g. where detection of under or over-expression of the receptor is required). Further preferred aspects of the invention are indicated in the following patent claims.

Detailed Description

15 Isolation and Sequencing of VIP₂ receptor Gene Preparation of Rat Pituitary cDNA

Anterior pituitary glands from male rats (Cob Wistar, 250g) were removed and total RNA was isolated by the method of Chomczynski and Sacchi[47]. Single stranded cDNA synthesis 20 and PCR were carried out using a commercial kit (Perkin Elmer Cetus). 1µg RNA was annealed with 2.5µM random hexanucleotide primers by heating to 95°C for five minutes, then cooling to 4°C over fifteen minutes. Single stranded cDNA was synthesised by incubating the oligonucleotide-RNA 25 solution at 42°C for 15 minutes in 20µl 10mM Tris-HCl, pH 8.3, containing 50mM KCl, 5mM MgCl₂, 1mM each dNTP, 20 units RNase inhibitor and 50 units reverse transcriptase. The reaction was terminated by heating to 99°C for 5 minutes.

Selective Amplification of Rat Pituitary cDNA

30 PCR was performed using a pair of degenerate 32-mer oligonucleotide primers (Figure 3), corresponding to conserved regions in the third and seventh transmembrane domains of the rat secretin[13], pig calcitonin[18] and

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opossum parathyroid hormone PTH[19] receptors. In more detail, each "primer" comprises a mixture of a large number of oligonucleotides with different permutations of nucleotide at particular positions within the

5 oligonucleotide sequence corresponding to the codons for particular amino acids within the receptor peptide sequence coded for thereby. This is necessary in order to include different codons which code for the same amino acid due to the degeneracy of the genetic code. Alternative nucleotides

10 are indicated above each other. Where it is desired to include the possibility of any one of the A, G, C, and T nucleotides (containing the bases Adenine, Guanine, Cytidine, and Thymidine) then the I nucleotide (with the base Inosine) is used. Sequences containing restriction

15 sites (EcoRI and BamHI, respectively) were added to the ends of the two oligonucleotide primers. Sequences of the two oligonucleotides are shown, aligned with the corresponding amino acid sequences in the rat secretin, pig calcitonin and opossum PTH receptors in Fig.3.

20 Reactions (100 μ l) contained 30pmol of each primer, 20 μ l reverse transcriptase reaction and 2.5 units Amplitaq DNA polymerase in 50mM KCl, 10mM Tris-HCl, pH 8.3 and 2mM MgCl₂ 5 cycles of PCR (60s denaturation at 94°C, 60s annealing at 45°C, 60s extension at 60°C) were followed by a further 40

25 cycles (60s denaturation at 94°C, 60s + 6s per cycle annealing and extension at 60°C) followed by 7 minutes at 60°C. PCR products were precipitated with ammonium acetate and ethanol after which one half of each reaction was run on a 1% agarose gel. Five bands containing cDNA sequences

30 having a size corresponding generally to that expected of the cDNA within the receptor gene between the two primers used, and ranging in size from 500bp to 900bp were excised from the gel and purified using the Sephaglas BandPrep kit (Pharmacia) and one quarter was used for a

35 further round of PCR (5 cycles of 60s denaturation at 94°C, 60s annealing at 47°C, 60s extension at 60°C followed by a

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further 40 cycles of 60s denaturation at 94°C, 60s + 6s per cycle annealing and extension at 60°C followed by 7 minutes at 60°C.

Preparation of cDNA clones

5 PCR products were ethanol precipitated, digested with BamHI and EcoRI, separated on a 1% agarose/TAE gel, purified using the Sephaglas BandPrep kit (Pharmacia), ligated into pBluescript SK-Vector (Stratagene) and used to transform competent E. coli DS941. The sequences of several of the
10 clones were obtained on both strands (Sequenase 2.0 kit, USB). One of the clones (RPR4) was found to have substantial similarity to rat VIP₁ receptor[4, 5] but was distinct from all the known members of the family.

Tissue Distribution of mRNA (Northern Blotting)

15 Total RNA was isolated from tissues using the guanidinium thiocyanate/caesium chloride method[48]. Approximately 20µg of each RNA was separated by electrophoresis on denaturing 1% agarose/formaldehyde gels, transferred to a nitrocellulose membrane (Hybond-C, Amersham) and baked for
20 2h at 80°C. The membrane was then hybridised with the insert from RPR4 that had been labelled with [32P]dCTP-using random hexanucleotide primers (Pharmacia) and the Klenow fragment of E.coli DNA polymerase[49]. Hybridisation was performed overnight in 50% formamide, 25mM KP04, pH7.4, 5xSSC
25 (1xSSC=0.15M NaCl, 0.015 M Na citrate pH7.0), 5x Denhardt's solution, 50µg/ml salmon sperm DNA. They were then washed twice for 20 min in 2xSSC/0.1% SDS at 50°C, then twice more in 0.5xSSC/0.1% SDS at 50°C and exposed to Fuji RX film. This
30 Northern blot analysis using RPR4 as a hybridisation probe revealed a mRNA transcript of approximately 3.5 kilobases expressed in the pituitary and regions of the brain, with the highest levels being observed in the olfactory bulb.

Screening Rat Olfactory Bulb cDNA Library

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One of the clones isolated, RPR4, was used accordingly to screen a commercial cDNA library in the Lambda Zap II vector[50] prepared from rat olfactory bulb (Stratagene Catalogue No. 936520).

5 4×10^5 plaque forming units of the library (corresponding to one-fifth of the total number of 2×10^6 primary plaques) were plated onto 20x 140mm plates using *E.coli* XL1-blue as a host strain. Filters containing plaque lifts from these plates were denatured by submerging in 1.5M NaCl, 0.5M NaOH for 2
10 mins, followed by neutralization in 1.5M NaCl, 0.5M TrisHCl pH8.0 for 5 mins, then rinsed in 3xSSC. The filters were then blotted dry on Whatman 3MM filter paper and baked at 80°C for 2 hours. They were then hybridised with the insert from RPR4 labelled using the same method found in the
15 Northern Blot protocol as described hereinabove.

Hybridisation was performed overnight in 50% formamide, 6x SSPE, 5x Denhardt's, 0.5% SDS, 100µg/ml salmon sperm DNA at 45°C. Positive plaques were identified, picked, and the phage purified by further rounds of plating and screening.
20 This procedure yielded six positive clones, of which three were analysed further. Positive clones were excised by co-infecting *E.coli* strain XL-1 blue with ExAssist (TM) helper phage (Stratagene) Bluescript SK phagemids (packed as filamentousphage particles) were then used to generate
25 double-stranded plasmids by infecting *E.coli* strain SOLRTM, and plating on L-broth/ampicillin plates to produce colonies. The pBluescript SK-double-stranded plasmid was then recovered using standard techniques[51].

Three different clones were isolated and characterised by
30 restriction mapping and sequencing. All three had sequences corresponding to RPR4 but only one cDNA, RPR4/6.3 containing an insert of 3.3 kb, encoded a complete open reading frame, encoding a protein of 437 amino acids with a predicted molecular weight of 49519 (Figure 2). A 22 amino acid
35 hydrophobic signal sequence is found at the amino-terminal

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end, with a predicted signal cleavage site between Pro²² and Glu²³[52]. A hydropathy plot shows seven hydrophobic, putative membrane-spanning domains (Figure 2). Comparison with other members of the secretin/calcitonin/PTH receptor family (Figure 3) revealed that the predicted protein encoded by RPR4/6.3 has greatest similarity with the rat VIP₁ and PACAP type I receptors (50% identity, with each). The highest amino acid sequence identity is found in the putative transmembrane regions, whereas the sequences of the amino-terminal extracellular domains and the carboxyl-terminal cytoplasmic ends are highly divergent.

Expression of VIP₂ receptor Gene

In order to determine the pharmacological characteristics of the novel receptor, the insert from the full length clone (RPR4/6.3) was excised as an EcoRI fragment and ligated into the EcoRI site of the mammalian cell expression vector pcDNA-1 (Invitrogen)[53, 54], and transiently transfected into COS-7 cells.

COS 7 cells were grown in DMEM supplemented with 10% newborn calf serum and 100U/ml each of penicillin and streptomycin, in a humidified atmosphere of 95% air/ 5% CO₂ at a constant temperature of 37°C, and were passaged every 3-4 days. Cells for transfection were trypsinised the day before the experiment and plated at a density of approximately 40-50% confluency in 75cm² flasks.

For transfection, cells were washed twice with OptiMEM (Gibco) supplemented with 100U/ml each of streptomycin and penicillin at 37°C before exposure to transfecting medium for 4h. The transfecting medium consisted of OptiMEM/ penicillin/ streptomycin, 400µg/ml DEAE dextran (Promega), 100µM chloroquine phosphate (Sigma) and 10-20µg plasmid per flask. This was replaced with 10% DMSO in PBS for 2 min, then DMEM/ 2% UltraSer G/ penicillin/ streptomycin. Cells

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were grown for 24h, then trypsinised and re-plated. Cells were harvested 48h later.

Functional Assay of VIP₂ receptor Gene

- Since all members of the secretin receptor family so far
5 identified are associated with activation of adenylate cyclase (also referred to in the art as adenylyl cyclase) the transfected cells were stimulated with several potential ligands, and intracellular cAMP levels measured by radioimmunoassay.
- 10 For screening purposes, cells transfected with RPR4/6.3 were seeded onto 12-well tissue culture dishes, and for dose/response experiments, onto 24-well dishes. Prior to incubation with various peptide ligands, the cultures were washed with DMEM containing 0.25% BSA, and
15 pre-incubated at 37 C for 30 min in the presence of 0.5mM isobutyl methylxanthine (IBMX). VIP ligand and other peptides were directly added at concentrations indicated in Figs. 4a and 4b and incubated at 37 C for 15-30 min. The reaction was stopped by adding ice cold 0.1M HCl, and the
20 cells homogenised by trituration. The levels of cAMP in the acidic extracts were measured by radioimmunoassay using antiserum CAB4 (courtesy of K.J. Catt, NICHD, NIH, Bethesda, MD) [55].

Results

- 25 Fig. 4a shows the effect of stimulating cells transfected with RPR4/6.3 by various peptide ligands. Values of cAMP release are expressed (mean \pm SEM, n=3) as a percentage of the stimulation evoked by 100nM VIP. While treatment with corticotropin releasing factor (CRF), calcitonin gene
30 related peptide (CGRP), secretin and glucagon, exhibited negligible effect, treatment with VIP, PACAP27, and PACAP-38 resulted in a marked elevation of cAMP levels. PHI and rat GHRH (rGHRH) also stimulated cAMP levels but were significantly less potent. PHI, VIP and PACAP-38 failed to

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stimulate cAMP levels in a control experiment where COS-7 cells had been transfected with the 5HT_{1A} (Serotonin) receptor (data not shown).

As shown in Fig.4b the stimulation of cAMP levels by VIP, PACAP, helodermin, and PHI was dose-dependent. The EC₅₀ (peptide concentration for 50% of maximal effect) for cAMP accumulation was approximately 0.18nM for PACAP-38, 0.43nM for PACAP-27, 0.25nM for helodermin, 0.17nM for VIP and 2.14nM for PHI. (The values shown in Fig.4b are the mean of 3. Basal cAMP was 0.8±0.15 pmoles/well). The maximal stimulation of cAMP accumulation by rGHRH (400nM) was only 60% of that found for VIP. We therefore conclude that the order of potency of these ligands is VIP- PACAP38- PACAP27- helodermin >PHI >>rGHRH, and that accordingly, RPR4/6.3 encodes a high affinity receptor for VIP.

Description of the Figures

Fig. 1 shows the gene encoding the rat VIP₂ receptor. The polynucleotide sequences specifically elucidated thus far are indicated along with the deduced amino-acid sequence;

Fig. 2 shows schematically the 7 transmembrane domains of the rat VIP₂ receptor and the 4 extracellular and 4 intracellular domains of the amino acid sequence derived from RPR4/6.3 using standard single letter codes to represent the amino acids;

Fig.3 shows the sequences for the two degenerate oligonucleotide primers; and

Fig.4 shows cAMP accumulation monitored during functional assay of a cloned gene of the invention.

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CLAIMS

1. A gene encoding VIP₂ receptor.
2. A gene encoding VIP₂ receptor having the amino acid sequence of SEQ ID No.1.
- 5 3. An isolated DNA sequence encoding VIP₂ receptor.
4. A recombinant DNA sequence encoding VIP₂ receptor.
5. An isolated or recombinant DNA sequence for use in expression in a prokaryotic or eukaryotic host cell of a polypeptide product having an amino acid sequence
10 corresponding to a sufficient extent to that of a naturally occurring VIP₂ receptor to provide at least one biological function of said naturally occurring VIP₂ receptor, which DNA sequence is selected from:
 - a) the DNA sequence of SEQ ID No. 1 or a sequence
15 complementary thereto; and
 - b) a DNA sequence which can hybridize to a DNA sequence defined in (a) or to a fragment thereof.
6. A gene or sequence according to any one of claims 1,3,4 and 5b wherein said VIP₂ receptor is human VIP₂ receptor.
- 20 7. A gene or sequence according to claim 6 which has been obtained directly or indirectly through recovery of a human gene by hybridisation thereof with a polynucleotide probe comprising a labelled DNA or RNA sequence capable of specifically hybridizing to a gene according to claim 2.
- 25 8. A prokaryotic or eukaryotic host cell transformed or transfected with a gene or DNA sequence according to any one of the preceding claims.

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9. A recombinant vector containing a DNA sequence or gene according to any one of claims 1 to 7.
10. A prokaryotic or eukaryotic host cell transformed or transfected with a recombinant vector according to claim 9.
- 5 11. A polynucleotide probe comprising a labelled DNA or RNA sequence capable of specifically hybridizing to a gene according to claim 1, claim 2 or claim 6 when dependent on claim 1 or claim 2, or a naturally occurring variant thereof.
- 10 12. A method of detecting a gene according to claim 1 or claim 2 or claim 6 when dependent on claim 1 or claim 2, which method comprises hybridizing a probe according to claim 11 with said gene, and detecting bound labelled probe.
13. An antibody probe comprising a labelled antibody raised
15 against an amino acid sequence capable of specifically binding to VIP₂ receptor expressed by a gene according to claim 1 or claim 2 or claim 6 when dependent on claim 1 or claim 2, or a naturally occurring variant thereof.
14. An antibody probe according to claim 13 which is a
20 monoclonal antibody.
15. A method of detecting VIP₂ receptor expressed by a gene according to claim 1 or claim 2 or claim 6 when dependent on claim 1 or claim 2, or a naturally occurring variant thereof, which method comprises allowing a probe according
25 to claim 13 or claim 14 to bind with said receptor, and detecting bound labelled probe.
16. A method of diagnosis for a condition associated with VIP₂ receptor abnormality which comprises hybridizing a polynucleotide probe according to claim 11 with the 7q36.3

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chromosomal region, and monitoring for absence of bound labelled probe.

17. A method according to claim 16 wherein is used the technique of fluorescence in situ hybridisation.

5 18. A method of diagnosis for a condition associated with VIP₂ receptor abnormality which comprises amplification by polymerase chain reaction using polynucleotide primers from substantially spaced apart portions of a nucleic acid sequence encoding VIP₂ receptor, and monitoring for absence
10 of amplified polynucleotide sequence product.

19. A method of diagnosis for a condition associated with VIP₂ receptor abnormality which comprises microscopic examination of the chromosomal region for gross abnormality.

20. A method of diagnosis for holoprosencephaly comprising
15 the steps of collecting a foetal sample and examining the chromosomal content thereof by a method according to any one of claims 16 to 19.

21. A method of evaluating a potential VIP₂ agonist or antagonist comprising the steps of: providing cell membrane
20 of a VIP₂ receptor expression system comprising a transformed or transfected host cell according to claim 8 or claim 10, which membrane has VIP₂ receptor; exposing said cell membrane to labelled ligand and to said agonist or antagonist; and monitoring at least one of ligand binding
25 and an intra-cellular messenger system.

22. A method according to claim 21 which includes the preliminary step of providing a VIP₂ receptor expression system comprising a transformed or transfected host cell according to claim 8 or claim 10.

1/6FIG. 1

SEQ ID NO: 1
 SEQUENCE TYPE: Nucleotide with
 corresponding protein
 SEQUENCE LENGTH: 2128 base
 pairs

STRANDEDNESS: double
 TOPOLOGY: linear
 MOLECULE TYPE: cDNA TO mRNA

ORIGINAL SOURCE
 ORGANISM: rat
 IMMEDIATE EXPERIMENTAL
 SOURCE
 Olfactory bulb

FEATURES

from 69 to 134 bp signal peptide
 from 135 to 1382 bp mature peptide
 from 237 to 239 bp potential
 glycosylation site
 from 327 to 329 bp potential
 glycosylation site
 from 339 to 341 bp potential
 glycosylation site
 from 447 to 512 bp transmembrane
 domain 1 (putative)
 from 540 to 599 bp transmembrane
 domain 2 (putative)
 from 675 to 746 bp transmembrane
 domain 3 (putative)
 from 783 to 851 bp transmembrane
 domain 4 (putative)
 from 903 to 974 bp transmembrane
 domain 5
 from 1050 to 1109 bp transmembrane
 domain 6 (putative)
 from 1146 to 1205 bp transmembrane
 domain 7 (putative)

PROPERTIES: VIP2 receptor for
 vasoactive intestinal peptide
 (VIP)

CGCCTGGGAG	GCCCCGAGCT	GGCGTTACTG	CTGAGGGCGC	CAAGGACCGA	GGCGGCACTG	60
AGCCCCAGG	ATG AGG GCG TCG GTG GTG CTG ACC TGC TAC TGC TGG TTG CTG GTG	113				
	Met Arg Ala Ser Val Val Leu Thr Cys Tyr Cys Trp Leu Leu Val					
	-20 -13 -10					
CGG GTG AGC AGC ATC CAC CCA GAA TGC CGG TTT CAT CTG GAA ATA CAG GAA	164					
Arg Val Ser Ser Ile His Pro Glu Cys Arg Phe His Leu Glu Ile Gln Glu						
-5 -1 1 5 10						
GAG GAG ACA AAA TGT GCA GAG CTG CTA AGC AGC CAA ATG GAG AAT CAC AGA	215					
Glu Glu Thr Lys Cys Ala Glu Leu Leu Ser Ser Gln Met Glu Asn His Arg						
15 20 25						
GCT TGC AGC GGT GTC TGG GAC AAC ATC ACA TGC TGG CGC CCT GCA GAC ATT	266					
Ala Cys Ser Gly Val Trp Asp Asn Ile Thr Cys Trp Arg Pro Ala Asp Ile						
30 35 40						

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GGG	GAA	ACT	GTC	ACA	GTG	CCC	TGC	CCC	AAA	GTG	TTC	AGC	AAT	TTC	TAC	AGC	317
Gly	Glu	Thr	Val	Thr	Val	Pro	Cys	Pro	Lys	Val	Phe	Ser	Asn	Phe	Tyr	Ser	
45					50					55					60		
AGA	CCA	GGA	AAC	ATA	AGC	AAA	AAC	TGC	ACT	AGT	GAT	GGG	TGG	TCG	GAG	ACA	368
Arg	Pro	Gly	Asn	Ile	Ser	Lys	Asn	Cys	Thr	Ser	Asp	Gly	Trp	Ser	Glu	Thr	
			65					70					75				
TTT	CCG	GAT	TTC	ATA	GAT	GCG	TGT	GGC	TAC	AAC	GAC	CCC	GAG	GAT	GAG	AGT	419
Phe	Pro	Asp	Phe	Ile	Asp	Ala	Cys	Gly	Tyr	Asn	Asp	Pro	Glu	Asp	Glu	Ser	
80						85					90					95	
AAG	ATC	ACG	TTT	TAT	ATT	CTG	GTG	AAG	GCC	ATT	TAT	ACC	TTG	GGC	TAC	AGT	470
Lys	Ile	Thr	Phe	Ile	Ile	Leu	Val	Lys	Ala	Ile	Tyr	Thr	Leu	Gly	Tyr	Ser	
				100					105					110			
GTT	TCT	CTG	ATG	TCT	CTT	ACA	ACA	GGA	AGC	ATA	ATT	ATC	TGC	CTC	TTC	AGG	521
Val	Ser	Leu	Met	Ser	Leu	Thr	Thr	Gly	Ser	Ile	Ile	Ile	Cys	Leu	Phe	Arg	
		115					120						125				
AAG	CTG	CAC	TGC	ACA	AGG	AAC	TAC	ATC	CAC	CTG	AAT	CTG	TTC	CTC	TCC	TTC	572
Lys	Leu	His	Cys	Thr	Arg	Asn	Tyr	Ile	His	Leu	Asn	Leu	Phe	Leu	Ser	Phe	
130					135					140					145		
ATG	CTG	AGA	GCC	ATC	TCT	GTG	CTG	GTC	AAG	GAC	AGT	GTG	CTC	TAC	TCC	AGC	623
Met	Leu	Arg	Ala	Ile	Ser	Val	Leu	Val	Lys	Asp	Ser	Val	Leu	Tyr	Ser	Ser	
			150					155					160				
TCA	GGT	ACA	CTG	CGC	TGC	CAC	GAC	CAG	CCG	GGC	TCC	TGG	GTT	GGC	TGC	AAG	674
Ser	Gly	Thr	Leu	Arg	Cys	His	Asp	Gln	Pro	Gly	Ser	Trp	Val	Gly	Cys	Lys	
165					170					175					180		
CTC	AGC	CTG	GTA	TTC	TTC	CAG	TAC	TGT	ATC	ATG	GCG	AAC	TTC	TAC	TGG	CTT	725
Leu	Ser	Leu	Val	Phe	Phe	Gln	Tyr	Cys	Ile	Met	Ala	Asn	Phe	Tyr	Trp	Leu	
				185					190						195		
CTG	GTG	GAG	GGT	CTC	TAC	CTG	CAC	ACC	CTC	CTG	GTA	GCC	ATC	CTT	CCT	CCC	776
Leu	Val	Glu	Gly	Leu	Tyr	Leu	His	Thr	Leu	Leu	Val	Ala	Ile	Leu	Pro	Pro	
		200					205					210					
AGC	AGG	TGT	TTC	CTG	GCC	TAC	CTT	CTT	ATT	GGA	TGG	GGT	ATC	CCC	AGT	GTG	827
Ser	Arg	Cys	Phe	Leu	Ala	Tyr	Leu	Leu	Ile	Gly	Trp	Gly	Ile	Pro	Ser	Val	
215					220				225						230		
TGT	ATA	GGT	GCA	TGG	ATA	GCA	ACT	CGC	CTT	TCT	TTA	GAA	GAC	ACA	GGT	TGC	878
Cys	Ile	Gly	Ala	Trp	Ile	Ala	Thr	Arg	Leu	Ser	Leu	Glu	Asp	Thr	Gly	Cys	
			235				240						245				
TGG	GAC	ACG	AAC	GAC	CAC	AGC	ATC	CCC	TGG	TGG	GTC	ATT	CGG	ATG	CCC	ATT	929
Trp	Asp	Thr	Asn	Asp	His	Ser	Ile	Pro	Trp	Trp	Val	Ile	Arg	Met	Pro	Ile	
250					255						260					265	
CTA	ATT	TCT	ATT	GTA	GTC	AAC	TTT	GCC	CTC	TTC	ATC	AGC	ATT	GTA	AGG	ATC	980
Leu	Ile	Ser	Ile	Val	Val	Asn	Phe	Ala	Leu	Phe	Ile	Ser	Ile	Val	Arg	Ile	
				270					275					280			
TTA	CTT	CAG	AAG	CTA	ACT	TCT	CCA	GAT	GTT	GGT	GGC	AAT	GAC	CAG	TCA	CAG	1031
Leu	Leu	Gln	Lys	Leu	Thr	Ser	Pro	Asp	Val	Gly	Gly	Asn	Asp	Gln	Ser	Gln	
		285					290					295					
TAC	AAG	AGG	CTC	GCC	AAG	TCC	ACA	CTG	CTG	CTA	ATC	CCA	CTG	TTT	GGC	GTC	1082
Tyr	Lys	Arg	Leu	Ala	Lys	Ser	Thr	Leu	Leu	Leu	Ile	Pro	Leu	Phe	Gly	Val	
300					305					310					315		

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CAC TAC ATG GTG TTT GCT GCC TTC CCC ATT GGC ATC TCC TCC ACG TAC CAG His Tyr Met Val Phe Ala Ala Phe Pro Ile Gly Ile Ser Ser Thr Tyr Gln 320 325 330	1133
ATC CTG TTT GAG TTA TGT GTT GGT TCC TTC CAG GGC CTG GTG GTC GCA GTT Ile Leu Phe Glu Leu Cys Val Gly Ser Phe Gln Gly Leu Val Val Ala Val 335 340 345 350	1184
CTA TAT TGC TTT CTG AAC AGT GAG GTA CAG TGT GAA CTG AAA AGA AGG TGG Leu Tyr Cys Phe Leu Asn Ser Glu Val Gln Cys Glu Leu Lys Arg Arg Trp 355 360 365	1235
AGA GGC CTG TGC CTG ACC CAG CCT GGG AGC CGG GAC TAC CGG CTG CAC AGC Arg Gly Leu Cys Leu Thr Gln Pro Gly Ser Arg Asp Tyr Arg Leu His Ser 370 375 380	1286
TGG TCC ATG TCC CGG AAT GGC TCA GAA AGC GCC CTA CAG ATA CAC CGT GGC Trp Ser Met Ser Arg Asn Gly Ser Glu Ser Ala Leu Gln Ile His Arg Gly 385 390 395 400	1337
TCC CGT ACC CAG TCC TTC CTG CAG TCA GAG ACC TCA GTC ATT TAGCTGTGTC Ser Arg Thr Gln Ser Phe Leu Gln Ser Glu Thr Ser Val Ile 405 410	1389
CCTCATAACAG AGCTGACAGT GCTGCTGGGT TTGACATATG TGTTCGCCAG CTTCTCTGTC TTGCCCCAGT GTCTGGTGCC TTATTGGGTC AGCCCTGGTC CTTAACTCGA TTGTAACTTG ATTGAAACAC CAGTTATTGT TGACAGACTC TAGCCTTTAA GCCATCCTCT TCATAATATG GCACAGCCAT ATTCTACTTT CAAAGAGAGC AAGGAAACCA GGTGGCCCTG AACATCAAAA CTGGGTCTTA GAACGTCCGA AAAAAACAA GGAGGAAGAT TCCAGTTTCT CCACTGCCTC CTGTCAAGGA GAACACGTC CAGAACCAGC TGAGCATCAC CACCCTGTGG CCCAGCGCAT GCCTATGGTT GTCTCTGACA TTCCTCTCAA TCTATGGCAT ACCAGGGAAA GTGGATGAAA GCAACCTTCT CATCTACAA GCTTCCTGGT GCCCCCGGCC TTGAGTTCTG TCCTGTGGAC TACATCTGGA CAGCCACACC CTTGCCATT CTTGGACCAT CTGGTAGTGA CCAAAGATAA GTCAGGGAGG ATATTCTATG TTCAGCGGCC AAGCCAGGAA CTCATCTCTT CCCAGGGCAC TTAGCATCTG CTGCTTCCTA CAAGTGGTGA GCAGCCCTGG ATCCCAGGCC GGCTGCTGGC ATGAAGGCC TCATTCATGA CTTGTCCATT CAGCCCAAGG TTGTTCTCTA GCTGGTAGAA AAACTTCGAT TTTTATT	1449 1509 1569 1629 1689 1749 1809 1869 1929 1989 2049 2109 2126

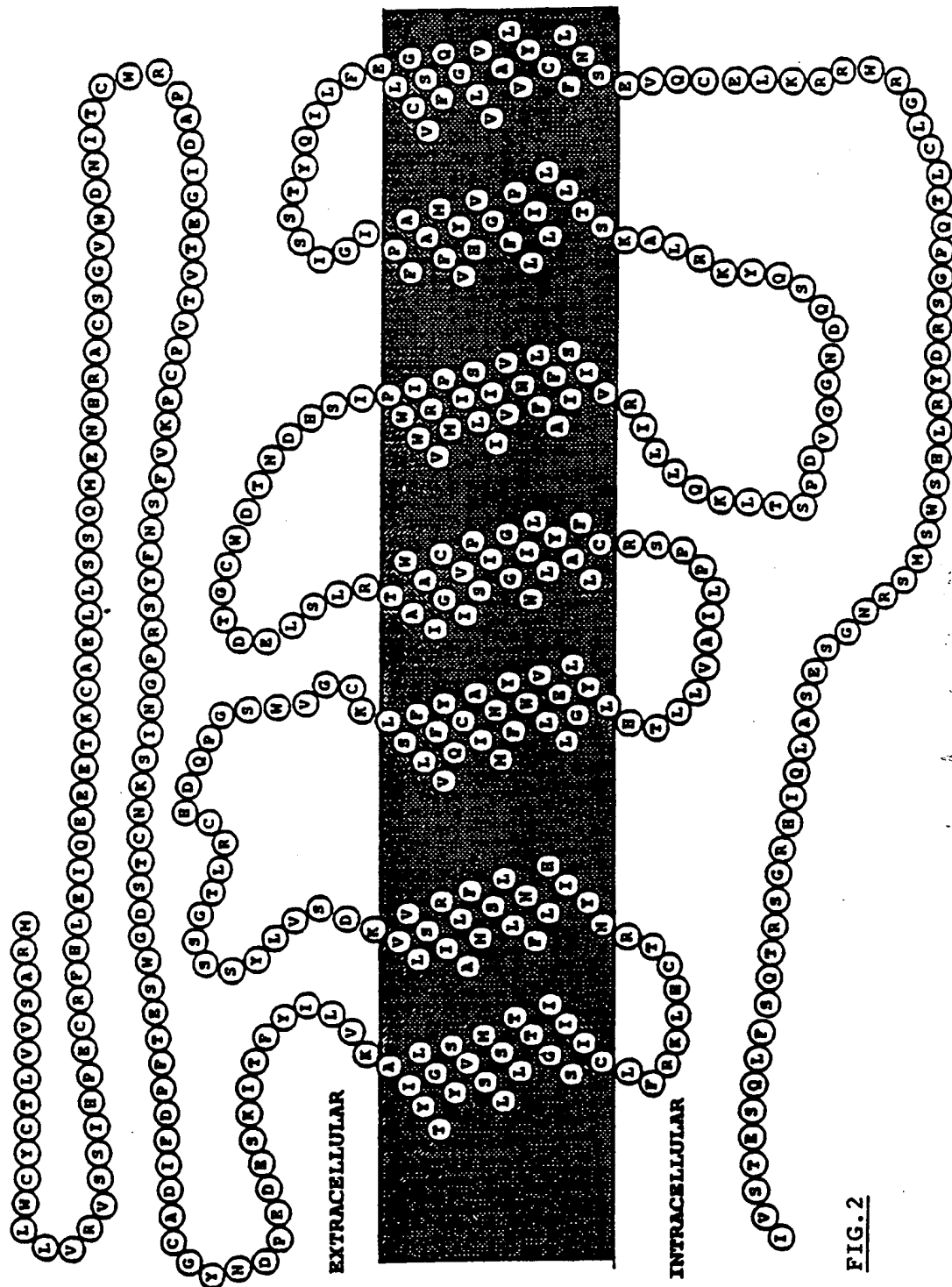


FIG. 2

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Calcitonin Asn Tyr Phe Trp Met Leu Cys Glu Gly Val Tyr

Parathyroid hormone Asn Tyr Tyr Trp Ile Leu Val Glu Gly Leu Tyr

Secretin Asn Tyr Ala Trp Leu Leu Val Glu Gly Leu Tyr

Oligo 1 5'-GGGAATTC AA_T^C TA_T^C G_T^A TGG A_T^C C_T^T GG_T^T GA_G^A GG_T^C G_T^C TA-3'

(Third Transmembrane)

Calcitonin Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys

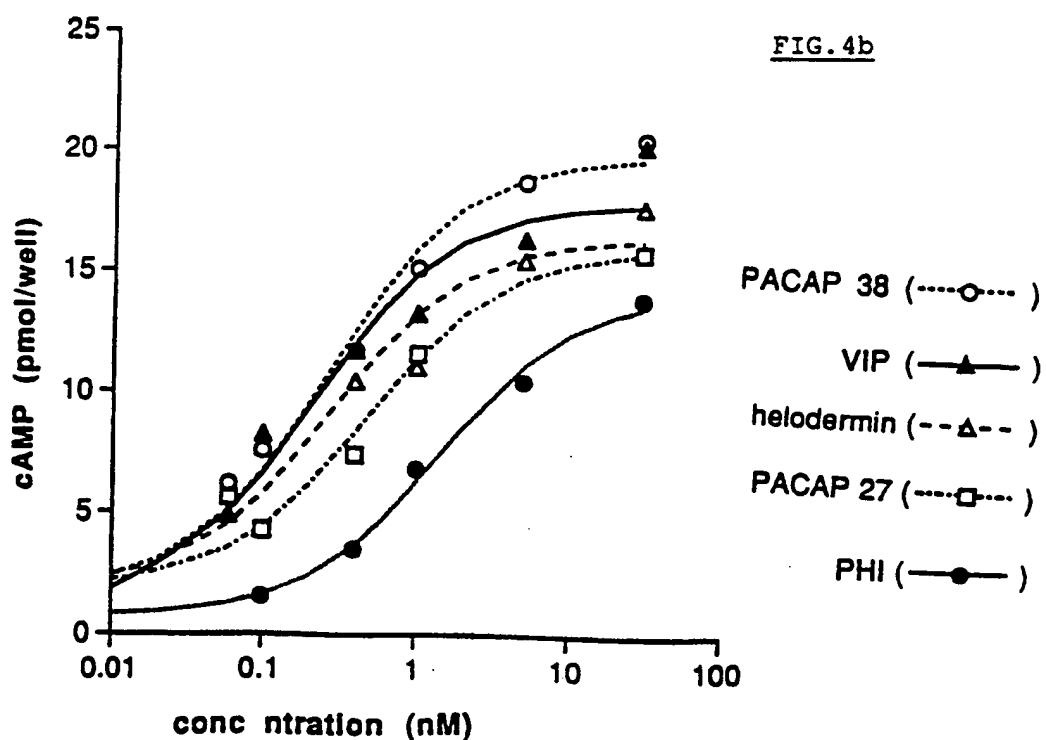
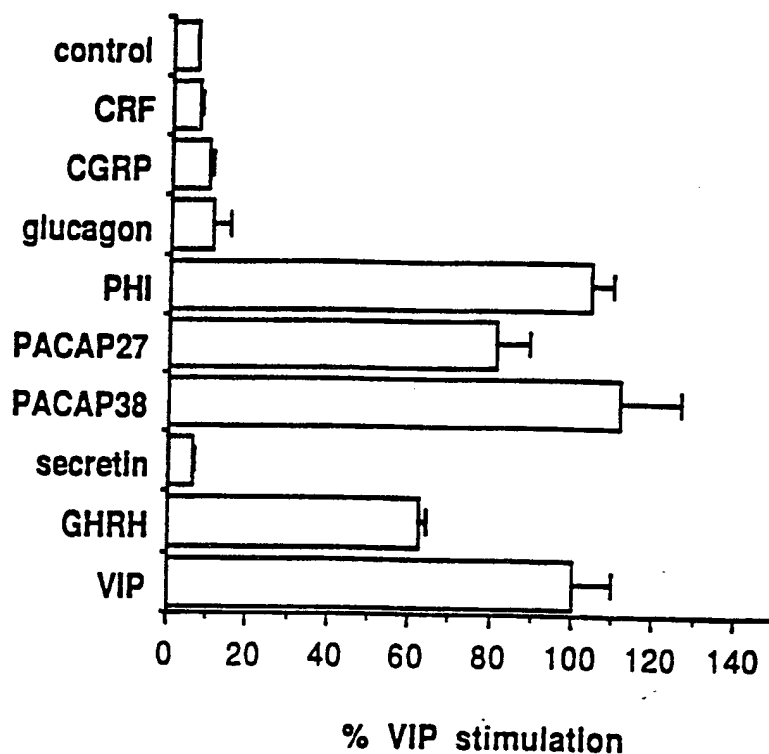
Parathyroid hormone Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys

Secretin Phe Gln Gly Leu Val Val Ala Val Leu Tyr Cys

Oligo 2 3'- AA_G^A GT_T^C CC_T^T GA_T^A CA_T^A CA_T^C CA_T^C CA_T^A CA_T^A AT_G^A AC CCTAGGGG-5'

(Seventh Transmembrane)

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/01892

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/10 G01N33/68
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	FEBS LETTERS., vol.334, no.1, 8 November 1993, AMSTERDAM NL pages 3 - 8 LUTZ EM;SHEWARD WJ;WEST KM;MORROW JA;FINK G;HARMAR AJ; 'The VIP2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide.' see the whole document ---	1-22
X	WO,A,92 21754 (OSAK BIOSCIENCE KENKYUSHO) 10 December 1992 see figure 1 --- -/--	5-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

20 December 1994

Date of mailing of the international search report

30.12.94

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Authorized officer

Nauche, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 94/01892

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>DATABASE WPI Week 9344, Derwent Publications Ltd., London, GB; AN 93-348480 & JP, A, 5 255 394 (ZH OSAKA BIOSCIENCE KENKYUSHO) 5 October 1993 see abstract</p> <p>-----</p>	5-12

Question on patent family members

PC 94/01892

Form PCT/ISA/210 (patent family annex) (July 1992)